

Lung Oxidative and Nitrosative Changes in Type II Diabetic Male Wistar Rats Treated with Lauric Acid

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ABSTRACT

Background: Sustained hyperglycemia in diabetes has been reported to cause excessive production of reactive oxygen and nitrogen species that mediate oxidative and nitrosative stress in the lungs. Lauric acid was reported to possess antioxidant, anti-inflammatory and insulinotropic properties.

Methodology: This study was designed to investigate the effect of lauric acid on the activity of inducible nitric oxide synthase (iNOS), malondialdehyde (MDA) concentration and activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the lungs of type II diabetic male Wistar rats. Thirty male Wistar rats were used for this study. They were divided into six groups of five rats each: Groups 1 and 2 served as non-diabetic and diabetic untreated respectively, while Groups 3, 4, 5 and 6 were diabetic Wistar rats treated with 125 mg/kg, 250 mg/kg, 500 mg/kg lauric acid and 100 mg/kg metformin respectively.

Results: There was a significant decrease ($p \leq 0.05$) in the level of MDA and activity of iNOS in the lungs of type II diabetic male Wistar rats treated with lauric acid when compared to that of the diabetic untreated. The activities of SOD and CAT in the lungs of type II diabetic Wistar rats treated with lauric acid were significantly increased ($p \leq 0.05$) when compared to that of diabetic untreated.

Conclusion: Lauric acid ameliorates oxidative and nitrosative changes in the lungs of type II diabetic male Wistar rats.

Keywords: Oxidative stress, Hyperglycemia, Lungs, Lauric acid, Type II diabetes

INTRODUCTION

Diabetes mellitus is a multifactorial and heterogeneous metabolic disorder characterized by elevated blood glucose levels, associated with impaired metabolism of



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carbohydrates, proteins and lipids, resulting from defects in insulin secretion, insulin action or the coexistence of both mechanisms^[1]. Being a metabolic syndrome, diabetes mellitus results in complications of many organ systems of the body; one of such is the impaired function of the pulmonary system termed “diabetic pneumopathy” which is associated with reduction of effective gaseous exchange in the lungs^[2,3]. The pulmonary complications were reported to be glucotoxicity-induced, relating to chronic hyperglycemia^[4].

Sustained hyperglycemia produces excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) that cannot be overwhelmed by endogenous antioxidant defence system, resulting in chain reactions that damage the structures of DNA, lipids, and proteins^[5]. It has been reported that diabetic lung was associated with decreased activity of superoxide dismutase, while the contents of nitric oxide (NO) and malondialdehyde were significantly increased^[6]. Alterations of nitric oxide metabolism have been reported as a metabolic marker of pulmonary function in diabetic states^[7]. In diabetic lung, a higher concentration of NO is produced, particularly by an inducible isoform of nitric oxide synthase, together with its potent oxidative derivative peroxynitrite; which is formed via oxidation with superoxide radical, mediate oxidonitrosative damage in the lungs^[8, 9]. As NO and superoxide radical can react simultaneously to produce peroxynitrite that is very damaging, any evidence that NO concentration is elevated in the diabetic lung would suggest that NO/peroxynitrite pathway is a mechanism by which lung is injured^[10].

Lauric acid is a 12-carbon medium-chain saturated fatty acid, a major component of triglycerides, and an important ingredient in coconut, laurel, and

palm kernel oils^[11, 12]. In coconut oil, the principal fatty acid is lauric acid, which makes up 45-65% of the oil, and has been related to several health-promoting benefits of coconut oil intake^[13]. A study reported that lauric acid was able to decrease NADPH-derived superoxide ion levels in the heart and kidney from spontaneously hypertensive rats^[14]. Although the mechanism of action of lauric acid on superoxide ions is unclear, it means that lauric acid may have the ability to ameliorate oxidative stress. This study was therefore aimed at investigating the effect of lauric acid on the levels of iNOS, MDA, SOD and |CAT in the lungs of type II diabetic male Wistar rats.

MATERIALS AND METHOD

Equipment, Drugs and Reagents

Digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany), Lauric acid (CAS-NO:143-07-7), Streptozotocin (STZ) (Sigma Aldrich, USA), Simas Margarine (PT Salim IvomasPratamaTbk, Indonesia), NOS2/iNOS Rats Enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Fine Biotech Co. Ltd China), Tween 80, Fructose (20% w/v) (Kem Light Laboratories PVT Ltd, India), Metformin (Jiangsu RuinianQianjin Pharmaceutical Co. Ltd, China). Vital feed (Grand cereals Ltd, Jos, Plateau State, Nigeria). Groundnut powder and groundnut oil (Samaru Market, Zaria, Kaduna State, Nigeria). All other chemicals used were of analytical grade.

Experimental Design

Thirty (30) male Wistar rats weighing about 80-100 g, were sourced from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The Wistar rats were housed in white transparent plastic cages and fed on standard commercial feeds with access to water *ad libitum* and allowed to

acclimatize for a period of two (2) weeks before the commencement of the research. The experiment was carried out in the Animal House of Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria. It was conducted following the guideline for use of animals in scientific research with approval number (ABUCAUC/2018/071), obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

Induction of Type II Diabetes Mellitus

Type II diabetes was induced by feeding the Wistar rats with a high-fat diet (HFD), after which a low dose of streptozotocin (STZ) was administered to them according to the method described by Okoduwa^[15], with slight modifications. A high-fat diet was prepared by mixing 10 grams of normal animal feed with 2.5 grams Simas margarine, 2.5 grams of groundnut powder and 1 gram of groundnut oil. The nutrient composition of the prepared HFD was 41% fats, 22% proteins and 37% carbohydrates. The animals were fed with the HFD along with 20% fructose (w/v) as drinking water for a period of six weeks. The Wistar rats were then injected with a single intraperitoneal dose of STZ at 40 mg/kg, diluted in 0.1 M citrate-buffered saline (pH 4.5), after overnight fasting. During the first 24 hours after STZ injection, 5% glucose (w/v) was provided as drinking water to the Wistar rats. Diabetes was confirmed by determining blood glucose concentrations 72 hours after STZ administration, while the validation of diabetes was done one week after initial confirmation, and rats with fasting blood glucose levels ≥ 300 mg/dl were considered diabetic.

Animal Grouping

After the induction of type II diabetes mellitus, the rats were divided into six (6) groups of 5 rats each. Lauric acid was dissolved in tween 80 (vehicle) at a ratio of 1:2 (w/v) and diluted to desired concentrations in distilled water. All administrations were done orally, once daily for a period of three (3) weeks.

Group 1: (Non-diabetic): Normoglycemic rats administered 1ml/Kg distilled water.

Group 2: (Diabetic untreated): Type II Diabetic rats administered 1ml/Kg tween 80

Group 3: Type II Diabetic rats administered 125 mg/kg of Lauric acid

Group 4: Type II Diabetic rats administered 250 mg/kg of Lauric acid

Group 5: Type II Diabetic rats administered 500 mg/kg of Lauric acid

Group 6: Type II Diabetic rats administered 100 mg/kg Metformin

Determination of Blood Glucose Level

Blood glucose levels were measured during confirmation and validation of diabetes, with blood samples obtained from Wistar rats' tail veins using a digital glucometer according to the glucose oxidase principle^[16].

Preparation of Lung Homogenate

At the end of the three (3) weeks treatment period, the rats were fasted overnight, and then anaesthetized with 50 mg/kg Ketamine hydrochloride. The thoracic wall was surgically opened; the lungs were removed, measured and homogenized with phosphate-buffered saline (KCl 140 mM, phosphate 20 mM, pH 7.4) at 9 ml per gram of lung tissue. The homogenate was centrifuged at $9000 \times g$ for 30 min^[17].

Biochemical Assays

An assay of inducible nitric oxide synthase activity was carried out using rat NOS2/iNOS (Nitric oxide synthase 2, inducible) ELISA kit according to the manufacturer's instruction.

Malondialdehyde (MDA) concentration, as a marker of lipid peroxidation in the lung homogenate, was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS) according to the doubling heating method^[18]. Antioxidant enzyme superoxide dismutase (SOD) activity in the lungs was determined spectrophotometrically, based on the principle of superoxide inhibition of auto-oxidation rate of hematoxylin^[19], while assay of catalase (CAT) in the lungs was done according to the method described^[20].

Statistical Analysis

All results were expressed as mean \pm Standard Error of Mean (SEM). Statistical significant differences between groups were assessed using one-way analysis of variance (ANOVA), which was followed by a Tukey's *post-hoc* test. In all cases, values of $p \leq 0.05$ were considered statistically significant. Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp. Armonk, USA) was used for the analysis.

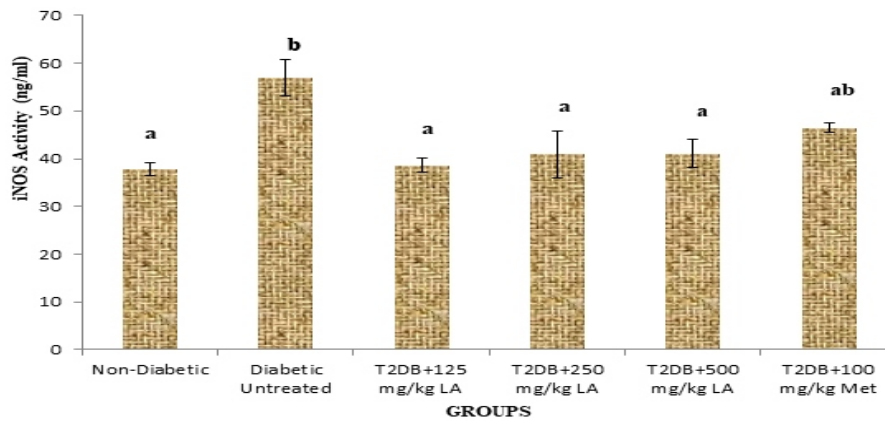
RESULTS

The result in figure 1 shows the effect of lauric acid on the mean activity of iNOS in the lungs of type II diabetic male Wistar rats. The mean iNOS activity in diabetic untreated rats was significantly increased at $p \leq 0.05$ compared to the non-diabetic rats. There was a significant decrease ($p \leq 0.05$) in iNOS activities and in the diabetic rats treated with 125 mg/kg, 250 mg/kg and 500 mg/kg lauric acid respectively, compared to the diabetic untreated

rats.

The result in figure 2 shows the effect of lauric acid on the mean concentration of MDA in the lungs of type II diabetic male Wistar rats. The concentration of MDA in diabetic untreated rats was significantly increased at $p \leq 0.05$ compared to the non-diabetic rats. However, after treatment with lauric acid, there was a significant decrease ($p \leq 0.05$) in concentrations of MDA in the diabetic rats treated with 250 mg/kg, 500 mg/kg lauric acid and 100 mg/kg metformin respectively, compared to the diabetic untreated rats.

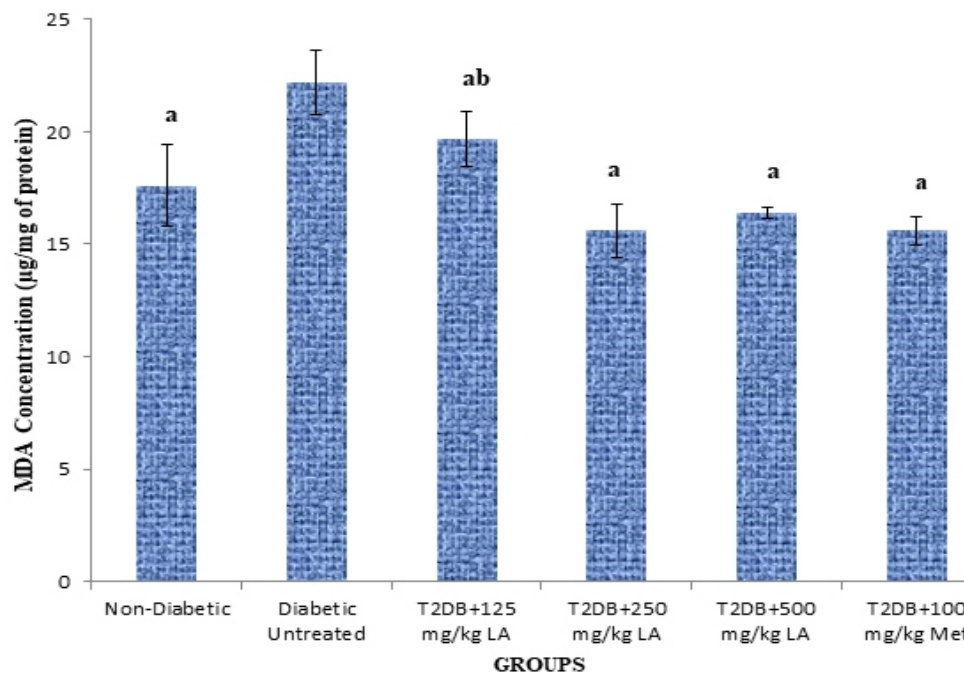
Figure 3 shows the result on the effect of lauric acid on the mean activities of SOD and CAT in the lungs of type II diabetic male Wistar rats. The SOD activity in diabetic untreated rats was significantly decreased at $p \leq 0.05$ compared to the non-diabetic rats. There was a significant increase ($p \leq 0.05$) however, in SOD activity in the diabetic rats treated with 500 mg/kg lauric acid compared to the diabetic untreated. The SOD activity in the diabetic rats treated with 100 mg/kg metformin was significantly reduced ($p \leq 0.05$) when compared to non-diabetic, but not significant when compared to diabetic untreated. The CAT activity in diabetic untreated rats was significantly decreased at $p \leq 0.05$ compared to the non-diabetic rats. There was a significant increase ($p \leq 0.05$) in CAT activity in the



diabetic rats treated with 125 mg/kg and 500 mg/kg lauric acid respectively, compared to the diabetic untreated rats.

Figure 1: Effect of lauric acid on the mean activity of inducible nitric oxide synthase (iNOS) in the lungs of type II diabetic male Wistar rats

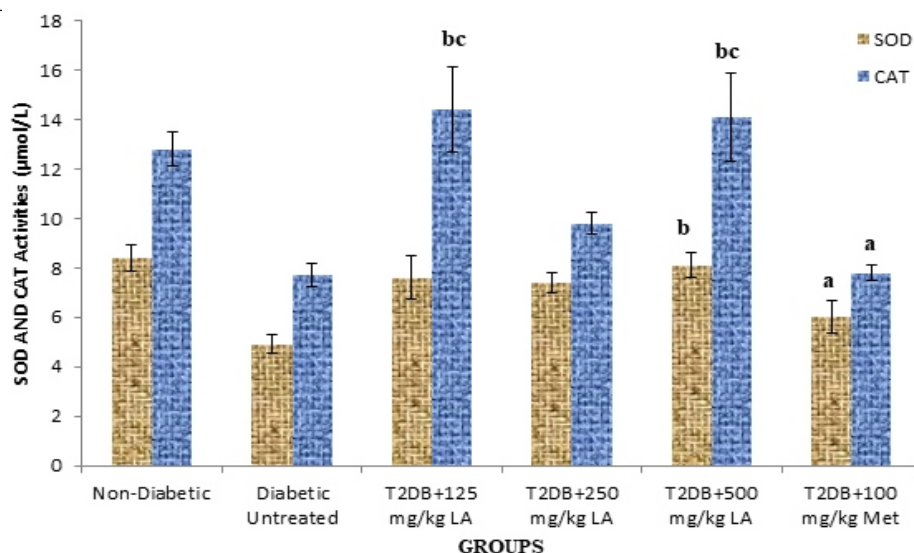
Data were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test; and results are presented as



mean \pm standard error of the mean of five Wistar rats. Bars with different superscripts are statistically significant at $p \leq 0.05$. LA= lauric acid, T2DB= Type II Diabetes, Met= Metformin

Figure 2: Effect of lauric acid on the mean malondialdehyde (MDA) concentration in the lungs of type II diabetic male Wistar rats

Data were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test; and results are presented



as mean \pm standard error of the mean of five Wistar rats. Bars with different superscripts are statistically significant at $p \leq 0.05$. LA= lauric acid, T2DB= Type II Diabetes, Met= Metformin

Figure 2: Effect of lauric acid on the mean activities of Superoxide dismutase (SOD) and Catalase (CAT) in the lungs of type II diabetic male Wistar rats

Data were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test; and results are presented as mean \pm standard error of the mean of five Wistar rats. Bars with superscripts are statistically significant at $p \leq 0.05$. a= when compared with non-diabetic, b= when compared to diabetic untreated, c= when compared to diabetic treated with 100 mg/kg Met, LA= lauric acid, T2DB= Type II Diabetes, Met= Metformin

DISCUSSION

There was a significant increase in the activity of inducible isoform of nitric oxide synthase (iNOS) in the lungs of diabetic rats in this study. This result is consistent with the findings of previous studies [21, 22], which reported that iNOS activity was significantly increased in the pulmonary tissue of diabetic rats. Inducible nitric oxide synthase, unlike the constitutive isoforms (nNOS and eNOS), is expressed in response to pathological stimuli which is regulated at a pre-translational

level and induced by activated macrophages and proinflammatory cytokines such as tumour necrosis factor (TNF)- α , interferon (INF)- γ and interleukin(IL)-1 β , in various lung disorders [23,24,25]. Increased activity of iNOS, leads to excessive production of nitric oxide (NO) [26,27]. Unlike the NO produced from constitutive isoforms of NOS under physiologic conditions, the NO from iNOS changes into a cytotoxic agent and mediates actions that are independent of guanylate cyclase and

cyclic guanosine monophosphate (cGMP) [28, 29]. This mechanism may involve inhibition of DNA synthesis by inactivation of ribonucleotide reductase, massive loss of intracellular iron with alteration of ferritin and transferrin receptors function, inhibition of -Fe-S group-containing mitochondrial enzymes and metabolic disturbances such as inhibition of respiratory chain and Krebs' cycle [23,30]. Nitric oxide has one unpaired electron in its single orbit which can exist independently as a free radical, thereby undergoing a chemical reaction that involves a transfer of a single electron leading to the generation of more free radicals and other species called reactive nitrogen species (RNS); which form part of a propagative chain reaction [26, 31, 32]. Nitric oxide synthase, may sometimes undergo structural changes, leading to electron transfer between substrates, enzyme cofactors and products becoming 'uncoupled' so that electrons are transferred to molecular oxygen, leading to the synthesis of superoxide ion (O_2^-) rather than NO [32, 33, 34]. In the present study, there was an increase in the concentration of malondialdehyde (MDA) but a decrease in the activities of superoxide dismutase (SOD) and catalase (CAT) in the lungs of the diabetic rats. This result agrees with previous works that reported an increase in MDA, a marker of lipid peroxidation and a decrease in antioxidant enzymes of the lungs, SOD and CAT, which are markers of oxidative stress in the lungs induced by hyperglycemia [22,35]. Autoxidation of glucose leads to the production of reactive oxygen species (ROS), superoxide anions (O_2^-) [29]. Excess superoxide ion (O_2^-) reacts with NO from iNOS, to form a strong, stable oxidant called peroxynitrite ($ONOO^-$) [36]. The protonated form of peroxynitrite decomposes to form nitrate through the intermediate formation of OH and NO_2 [37]. These

species may interact with each other or oxygen and other ROS, forming higher oxides of nitrogen to mediate oxido-nitrosative damage in the lungs [38].

The ROS/RNS formed, cause damage to cellular macromolecules via peroxidation of lipids, particularly, the polyunsaturated fatty acids (PUFAs) moiety of membrane phospholipids. The process of lipid peroxidation involves the abstraction of a hydrogen atom from membrane PUFAs, and the generation of lipid peroxidation products, malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) and conjugated dienes (CD) [31, 39, 40]. Superoxide dismutase is a first-line antioxidant enzyme that catalyzes the dismutation of superoxide anion into hydrogen peroxide, while catalase breaks down the excess hydrogen peroxide produced from the dismutation of superoxide ions into water and oxygen [40, 41]. Elevation of superoxide anion and other ROS/RNS in diabetic lungs may cause an increase in the activities of SOD and CAT, as a compensatory response to scavenge these free radicals and to prevent oxidative stress. However, oxidative stress occurs due to an imbalance in the oxidants/antioxidants ratio, in favour of the oxidants [37]. Thus, the activities of the antioxidant enzymes SOD and CAT are reduced in oxidative stress as seen in this study.

Lauric acid treatment caused a significant reduction in the activities of iNOS, the concentration of MDA and increased activities of SOD and CAT in the lungs of diabetic rats. The mechanism of iNOS suppression by lauric acid may be due to inhibition of NF- κ B by binding to peroxisome proliferator-activated receptor- γ . Nuclear factor-kappa B activation increases the production of proinflammatory cytokines and consequently causes induction of inducible isoform of nitric oxide synthase (iNOS), resulting in increased ROS/RNS generation [42, 43]. Therefore,

by exerting anti-inflammatory action on NF- κ B through PPAR- γ , lauric acid may inhibit the activity of iNOS and reactive oxygen and nitrogen species, causing a reduction in oxidative and nitrosative stress in the diabetic lungs. Lauric acid may also, through PPAR- γ , mediate cellular antioxidant and anti-inflammatory effects by enhancing the transcription of anti-inflammatory and antioxidant genes, including nuclear factor-erythroid 2 related factor 2 (Nrf-2), that plays a crucial role in the pulmonary defence against oxidative stress^[37, 42, 44]. Lauric acid was reported to have reduced the level of NADPH-oxidase -derived superoxide ions^[14]. Thus, by decreasing the level of superoxide ion, lauric acid was able to decrease lipid peroxidation in the lungs due to a decrease in the concentration of MDA, and also increase the endogenous antioxidant defence system of the lungs, characterized by increased activities of SOD and CAT. All the three given doses of lauric acid reduced the activity of iNOS in the diabetic lungs, however, only 500 mg/kg was consistent in alleviating the oxidative stress status of the diabetic lungs. This study therefore, showed that lauric acid has the ability to reverse oxidative stress in diabetic lungs which was not previously explored.

Limitation of the study

There are no lungs histological photomicrographs, nitric oxide and reactive oxygen/nitrogen species were not assessed. The duration of the study was short, and thus the long term effects of lauric acid on diabetic lungs were not explored.

CONCLUSION

Lauric acid ameliorate oxidative and nitrosative stress in the lungs induced by type II diabetes mellitus, by suppressing the activity of iNOS, lipid

peroxidation and also increasing the activities of lung tissue antioxidant enzymes. However, there is need to explore the effects of lauric acid on mitochondrial oxidative rate and directly measure the reactive oxygen species in diabetic lungs for a longer duration.

Conflict of interest

The authors declare no conflict of interest in this research work.

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